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Response to angiotensin inhibition in rats with sustained renovascular hypertension correlates with response to removing renal artery stenosis

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Purpose: **Sustained (late-phase) renovascular hypertension is associated with lower plasma renin activity than is the early phase. It is not clear to what extent this reduced plasma renin activity reflects diminished influence of the renin-angiotensin system. It also is not clear whether this change in the character of the disease influences the effectiveness of surgical removal of the renal artery stenosis in reversing hypertension. Using an animal model of sustained (≥10 weeks after renal artery clipping) two-kidney, one-clip renovascular hypertension, we hypothesized that the magnitude of the depressor response to selective angiotensin II receptor blockade with losartan would reflect the influence of the renin-angiotensin system on hypertension and enable us to predict the depressor response to subsequent surgical removal of the clip.**

Methods: **The left renal arteries of 20 male Sprague-Dawley rats weighing 150 to 200 gm were fitted with a silver clip (0.23 mm internal diameter). Systolic blood pressure was measured by means of tail-cuff plethysmography for 10 weeks. Rats were then given losartan orally (30 mg/kg a day) for 1 week while blood pressure was monitored. After an additional week to allow recovery, 13 rats underwent surgical unclipping, and seven underwent sham repair. Blood pressure again was monitored over the final week.**

Results: **All two-kidney one-clip rats had hypertension 10 weeks after clipping (mean systolic blood pressure 206 ± 10 mm Hg). Losartan decreased systolic blood pressure by 36 ± 6 mm Hg. The response was variable, ranging from 3 to 66 mm Hg, and overall blood pressure did not normalize (170 ± 8 mm Hg). Subsequent surgical unclipping decreased systolic blood pressure by 46 ± 9 mm Hg. Again the response was variable, ranging from 10 to 99 mm Hg, although overall blood pressure did not normalize (164 ± 7 mm Hg). The decrease in blood pressure after unclipping showed a high correlation with the blood pressure decrease after losartan administration (r = 0.861, p < 0.001). Resting plasma renin activity (before intervention) was 16 ± 4 ng angiotensin I per milliliter per hour and was not predictive of the response to either losartan or surgical unclipping. The rats subjected to sham operations had no statistically significant changes in blood pressure. Histologic evaluation showed patent renal arteries without appreciable stenosis or intimal hyperplasia after removal of the clips.**

Conclusions: **In sustained two-kidney, one-clip renovascular hypertension, the depressor response to angiotensin II receptor blockade is attenuated, suggesting that late-phase hypertension becomes increasingly angiotensin II-independent. In our model, the extent to which sustained renovascular hypertension becomes refractory to 7 days of angiotensin II blockade is highly predictive of the ultimate outcome of surgical repair of renal artery stenosis. (J Vasc Surg 1998;28:167-77.)**

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Renovascular hypertension affects approximately three million persons in the United States and is the most common cause of potentially curable secondary hypertension.[1,2] Despite improvements in recognition and diagnosis, renovascular hypertension remains an enigmatic clinical problem. Ascertaining who has renovascular hypertension and predicting a favorable response to operative or endovascular intervention remain imprecise.[3] Although surgical intervention carries an acceptable morbidity rate of 10% to 20% and a mortality of 2% to 6%, the likelihood of a complete cure of atherosclerotic renal arterial stenosis with secondary renovascular hypertension remains less than 60%.[4-7] More important, as many as 20% of patients may be exposed to the risk of operation with no derived benefit in the control of hypertension.[4-7] In this era of increasing endovascular intervention, a more precise means of diagnosing the condition and predicting the likelihood of beneficial therapeutic intervention would be useful.

Renovascular hypertension begins with physical impairment or obstruction of renal blood flow, resulting in renal hypoperfusion.[1,8] Typically this condition is caused by either stenosis or occlusion of the renal artery, most often from atherosclerosis or fibromuscular dysplasia.[1] Clinical data and studies in which modifications of the classic Goldblatt two-kidney, one-clip model of renovascular hypertension are used have suggested that renovascular hypertension appears to evolve over three distinct phases: the early or acute phase, a transitional phase, and a chronic or sustained late phase.[9,10] The early phase depends primarily on the renin-angiotensin system.[9,10] Reduced renal perfusion stimulates renin secretion through the renal baroreceptor mechanism, leading to an increase in plasma angiotensin II, which provokes systemic hypertension.[10,11] Plasma renin activity in the early phase typically may become 3 to 10 times higher than normal. In this early phase, hypertension can be quickly and completely reversed by means of pharmacologic inhibition of the renin-angiotensin system or surgical repair (or unclipping) of the renal arterial stenosis.[9,12]

In the second or transitional phase, angiotensin remains elevated and in addition to its direct vasoconstrictor effect may increase blood pressure through activation of secondary vasoconstrictors such as the endothelium-derived constricting factor thromboxane-endoperoxide.[13] Angiotensin II stimulation of aldosterone secretion also results in sodium and water retention and increased extracellular fluid volume, which may sustain hypertension and secondarily suppress renin release.[1] Thus the transitional phase is characterized by changes away from the predominant angiotensin dependence of the early phase.

The chronic or sustained late phase of renovascular hypertension is characterized by secondary structural damage to the kidneys, vasculature, and other organs. In contrast to the early phase, the influence of angiotensin on maintaining elevated blood pressure in chronic renovascular hypertension is not clear.[14] Plasma renin activity returns toward normal values, and elevated blood pressure no longer completely or consistently responds to short-term treatment with either angiotensin-converting enzyme (ACE) inhibitors or the angiotensin II antagonist saralasin, suggesting a diminished role for angiotensin in maintaining hypertension.[9,15] Hypertension is neither consistently nor predictably reversed with repair of the stenosis or even removal of the stenotic kidney.[16,17] Thus late-phase renovascular hypertension is defined as the stage at which secondary pathologic changes develop with inconsistent or absent blood pressure responses to inhibition or blockade of the renin-angiotensin system or surgical correction of renal arterial stenosis.

These observations suggest a progression from predominantly angiotensin-dependent hypertension to a complex disease increasingly dependent on factors other than angiotensin II and perhaps even independent of the stenotic kidney itself. Because of these characteristics, we hypothesized that the response to surgical removal of discrete renal artery stenosis would diminish as the cause transformed from the simple angiotensin II–dependent early phase of renovascular hypertension to the more complex and perhaps progressively angiotensin II–independent character evolving with this disease. We proposed that the depressor response to 7 days of selective angiotensin receptor blockade may be a useful indicator of how fully the disease has evolved from the early into the later phases, thereby making the success of surgical repair less assured. We tested this theory in a rat model of two-kidney, one-clip renovascular hypertension by comparing the blood pressure response to 7 days of constant administration of the selective angiotensin II AT-1 receptor antagonist losartan with the blood pressure response to subsequent surgical repair.

METHODS

Two-kidney, one-clip renovascular hypertension was induced in 20 male Sprague-Dawley rats. Thirteen rats were used in the experimental proto-

col, and seven were used as controls. The control sham protocol was identical to the experimental protocol except that the surgical procedure did not include repair of the stenosis by means of removal of the renal artery clip. All procedures were approved by the institutional animal care committee of Henry Ford Hospital.

Study phases

Induction of unilateral renal arterial stenosis and renovascular hypertension. Rats weighing 150 to 175 gm were anesthetized by means of injection of sodium pentobarbital (50 mg/kg Nembutal intraperitoneally; Abbott, Abbott Park, Ill.). Body temperature was maintained at 37° C with a heating module (Aquamatic K-20; American Hamilton, Cincinnati, Ohio) and blanket. Monitoring was conducted until the rats awoke from anesthesia. Under antiseptic conditions, the left renal artery was exposed through a retroperitoneal flank incision and carefully dissected free of the renal vein; the contralateral kidney was not disturbed. A silver clip with an internal diameter of 0.23 mm was placed around the left renal artery, causing moderate stenosis. The wound was closed with 3-0 silk sutures, and a mixture of collodion and iodine was applied to facilitate healing. After recovery the rats were returned to their cages, where they were maintained for 10 weeks so that the hypertension might evolve into the sustained late stage.[9]

Sustained renovascular hypertension was defined as the development of hypertension after clipping of the renal artery was maintained for 10 weeks or longer. This period equaled approximately 7% of the life span of a healthy, nondiseased rat. As each week of life of a rat approximates 6 months of human life, this time was believed to represent an adequately sustained-duration model of renovascular hypertension. During this time the rats were fed standard rat chow (22/5 rodent diet; Teklad, Bartonville, Ill.) and tap water ad libitum. Systolic blood pressure was monitored weekly by means of tail cuff plethysmography with a programmed electrosphygmomanometer (PE-300; Narco Biosystems, Houston, Tex., and dual-beam oscilloscope; Tektronix, Houston, Tex.). Hypertension was defined as sustained systolic blood pressure of 150 mm Hg or more.

Blood pressure response to sustained angiotensin II blockade with losartan. Beginning with the eleventh week after clipping, all rats were treated with the angiotensin II receptor blocker losartan (supplied by Merck, Rahway, N.J.). Each rat received 30 mg/kg losartan in the drinking water every day for 1 week.[18] Blood pressure was monitored with a tail cuff 12 hours after treatment was initiated and again at 2, 3, 5, and 7 days into losartan treatment.

Recovery from chronic losartan treatment. After 1 week, losartan treatment was discontinued, and a recovery period of 1 week allowed. On the basis of the half-life of losartan and its active metabolite,[19] 1 week was calculated to be adequate to eliminate the influence of losartan, representing 84 half-lives of losartan and 28 half-lives of its active metabolite.[20]

After the recovery period (beginning of the 13th week after clipping), rats underwent a second survival protocol consisting of pentobarbital anesthesia, measurement of plasma renin activity, surgical unclipping (or sham procedure), and arterial catheter placement. More specifically, the rats were again anesthetized with pentobarbital (as earlier), and a femoral vein catheter was inserted with 250 μl of blood collected after a stabilization period of 30 to 60 minutes. The sample was collected into potassium ethylene diamine tetraacetate (EDTA), separated by means of centrifugation, and frozen at –20° C for later analysis of plasma renin activity by means of radioimmunoassay with a Gammacoat kit (Incstar, Stillwater, Minn.) as adapted from the original method of Haber et al.[20] Under anesthesia the rat underwent surgical removal of the renal arterial clip.

Surgical repair of renal arterial stenosis by means of unclipping versus sham unclipping procedure. The renal arterial stenosis produced with the clip was repaired by means of surgical unclipping in 13 rats. The remaining seven rats (sham group) underwent the identical procedure except that the clip was manipulated but not removed. Anesthesia and preparation were the same as earlier with the addition of a femoral cutdown in the right hind limb for collection of blood and direct measurement of blood pressure. The femoral vessels were dissected, and catheters were placed and secured in both the artery and vein. Polyethylene 50 and polyethylene 10 tubing (Fisher Scientific, Chicago, Ill.) were used to catheterize the vein and artery, respectively. The venous line was used for blood sampling (as earlier), and the arterial catheter was used to monitor blood pressure. The arterial line was flushed with heparinized saline solution and tunneled beneath the skin to an exit portal between the scapulae. Blood pressure was recorded with a Statham pressure transducer (Vigo-Spectramed, Oxnard, Calif.) connected to a Gould chart recorder (Gould, Valley View, Ohio).

The clipped left renal artery was exposed by means of reopening the left flank incision and careful dissection free from the renal vein. The clip was cleaned of scar tissue and removed, care being taken not to traumatize the renal vessels or parenchyma. The wound was closed, and collodion and iodine were applied as earlier. For the seven rats subjected to sham procedures, the same protocol was followed, except the clip was only prepared for removal and manipulated but neither opened nor removed.

Rats in both groups were allowed to recover with the femoral cannula in place for at least 2 hours until they awoke from anesthesia. Blood pressure was recorded throughout both operation and recovery, after which the cannulae were flushed with heparinized saline solution and capped. Systolic blood pressure was measured both directly with the permanent subcutaneous femoral arterial line and indirectly with a tail cuff once a day for the first 3 days and then once every other day for a total of 7 to 14 days. There was excellent agreement between direct and indirect procedures. At the conclusion of the postsurgical period, the rats were killed with an intraperitoneal overdose of pentobarbital. The repaired renal arteries were gently probed with polyethylene 10 tubing to make sure they were patent and no physical resistance was present at the clip site. Renal arteries of randomly selected rats were harvested and subjected to histologic examination. Segments of each renal artery taken from the clip site and proximal to it were fixed with formalin, embedded in paraffin, and stained with hematoxylin and eosin. Multiple 4 to 5 μm sections were evaluated for luminal narrowing or occlusion and any pathologic changes in the arterial wall.

Exclusion criteria

All rats had to reach a systolic blood pressure of 150 mm Hg or more to be considered to have hypertension. On the basis of previous experience with two-kidney, one-clip hypertension of more than 10 weeks' duration, rats that responded to angiotensin blockade with an abrupt decrease in systolic blood pressure to less than normotensive levels were excluded. This small subpopulation (six rats not included among the 20 study animals) responded dramatically with a rapid decrease in blood pressure. Unlike the situation for the rats with early-phase angiotensin-dependent two-kidney, one-clip hypertension given losartan, if the drug was not immediately withdrawn from the excluded rats, they died within 1 to 2 days. This criterion for exclusion was established before the study began. Finally, any rats that exhibited bleeding from the renal artery during surgical unclipping were excluded because of the potential effect of hemorrhage and hypovolemia on renin secretion.

Statistical analysis

All data are presented as mean value ± 1 standard error. The data obtained were analyzed with Sigma Stat statistical software (Jandel, San Rafael, Calif.). This included descriptive statistics, both paired and unpaired Student *t* tests, linear regression, and Pearson correlation as appropriate.

RESULTS

A total of 32 male Sprague-Dawley rats were initially enrolled in the study and underwent left renal arterial clipping. Four died, presumably of complications of hypertension or malignant hypertension, during the initial 10-week observation period. Six were excluded because of an immediate, extreme drop in blood pressure to hypotensive levels in response to losartan. Of these six, three died before losartan was withdrawn from their drinking water, and three were immediately taken off losartan and survived. Two more rats were excluded because of excess bleeding at the clip site during surgical repair. Of the 20 rats that met the study criteria, 13 underwent successful unclipping, and 7 underwent the sham procedure.

Results by phase

Induction of unilateral renal artery stenosis and renovascular hypertension. Systolic blood pressure rose continuously after clipping for 7 weeks and then remained relatively stable (Fig. 1). All 13 rats in the experimental group had hypertension (systolic blood pressure ≥150 mm Hg) by 10 weeks after clipping. Systolic blood pressure varied from 150 to 255 mm Hg, with a mean value of 206 ± 10 mm Hg. All rats in the sham group also had hypertension 10 weeks after clipping; systolic blood pressure ranged from 165 to 250 mm Hg with a mean value of 207 ± 12 mm Hg.

Blood pressure response to sustained angiotensin II blockade with losartan. In the experimental group, the blood pressure response to 7 days of sustained angiotensin II inhibition varied considerably, and the magnitude of the response was independent of blood pressure before losartan treatment (blood pressure 10 weeks after renal arterial clipping and immediately before losartan administration) (Fig. 2). Mean systolic blood pressure dropped by 36 ± 6 mm Hg, from 206 ± 10 mm Hg (10 weeks after

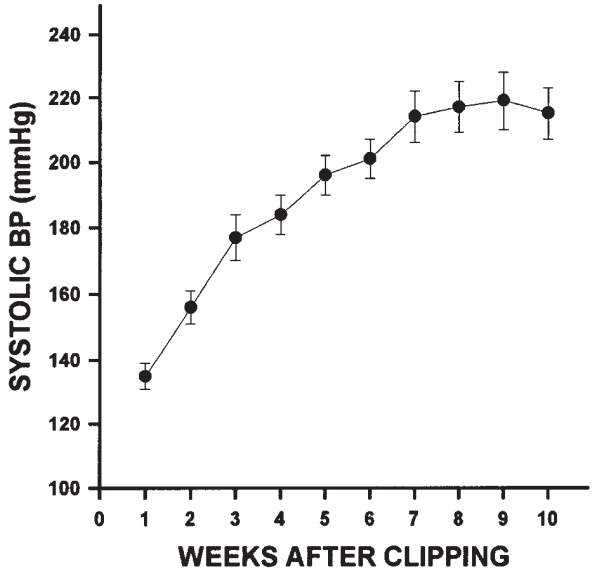

Fig. 1. Progression of systolic blood pressure over 10 weeks after clipping of the left renal artery in 20 rats.

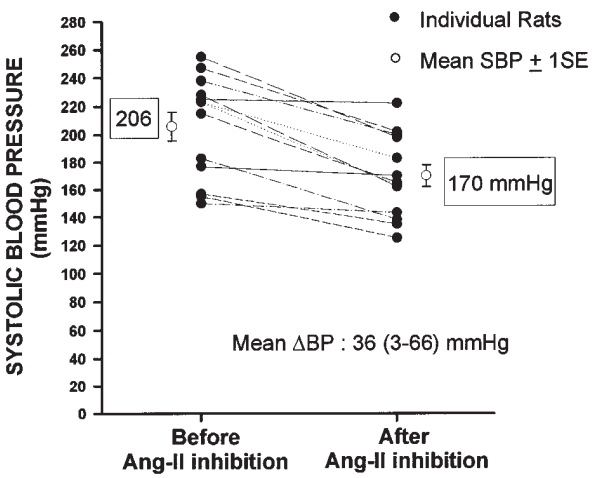

Fig. 2. Changes in systolic blood pressure induced by 1 week of sustained angiotensin receptor blockade by means of losartan among 13 rats with sustained (10 to 11 weeks) two-kidney, one-clip renovascular hypertension.

clipping) to 170 ± 8 mm Hg after administration of losartan ($p < 0.001$). The decrease in blood pressure in response to losartan varied from 3 to 66 mm Hg. The decrease in blood pressure with sustained losartan treatment exhibited a bimodal pattern. There was a relatively rapid decline over the first 12 hours of treatment, dropping some 30 ± 9 mm Hg, followed by a slower progressive decline over the next 2 days, achieving a mean decrease of 36 ± 6 mm Hg by day 3 (Fig. 3). Among the group subjected to sham procedures, systolic blood pressure response also varied with an overall systolic blood pressure decrease of 42 ± 11 mm Hg ($p < 0.01$) to a final blood pressure of 165 ± 4 mm Hg. The individual responses varied from 12 to 95 mm Hg. Thus rats in both experimental and sham groups had similar responses to losartan therapy. Overall, selective angiotensin blockade failed to restore blood pressure to previously normotensive values ($p < 0.004$).

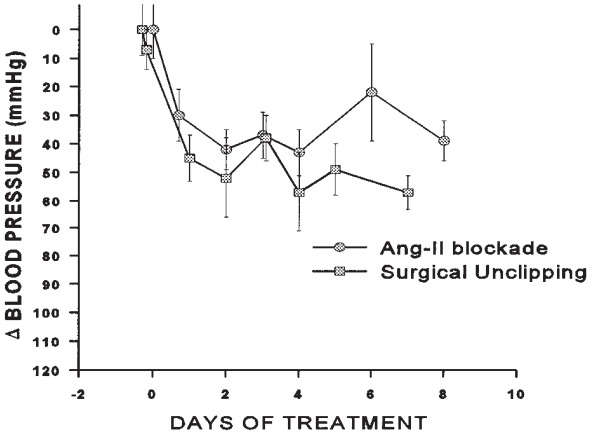

Fig. 3. Time course of the decrease in systolic blood pressure over 7 days in response to sustained losartan treatment and surgical repair among 13 rats with sustained two-kidney, one-clip renovascular hypertension.

Recovery from 7 days of losartan treatment. When losartan was withdrawn, systolic blood pressure returned to pretreatment values. After 7 days of recovery, mean systolic blood pressure had returned to 210 ± 9 mm Hg for the experimental group and 214 ± 9 mm Hg for the sham group, which correlated with paired pretreatment levels ($r = 0.94$ and 0.98, for experimental and sham groups, respectively; $p < 0.001$).

In the experimental group, mean plasma renin activity for anesthetized rats with two-kidney, one-clip hypertension 12 weeks after clipping was 16 ± 4 ng angiotensin I per milliliter per hour, and values varied from 0.92 to 55.5 ng angiotensin I per milliliter per hour. Plasma renin activity for the sham group was 11 ± 4 ng angiotensin I per milliliter per hour, no different from that of the experimental group.

Surgical repair of renal arterial stenosis by means of unclipping versus sham unclipping. Among the experimental group, surgical repair lowered systolic blood pressure by 46 ± 7 mm Hg (Fig.

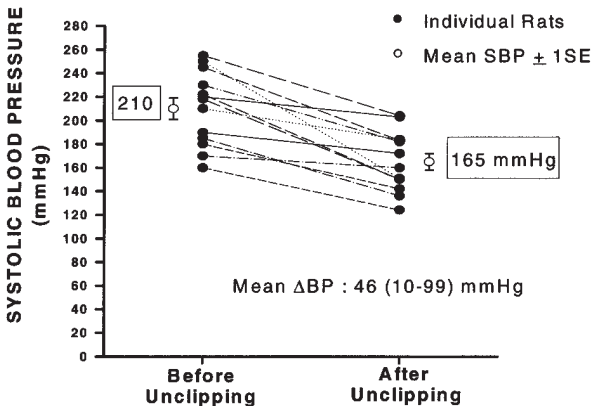

Fig. 4. Changes in systolic blood pressure in response to surgical repair of renal arterial stenosis among 13 rats with sustained two-kidney, one-clip renovascular hypertension.

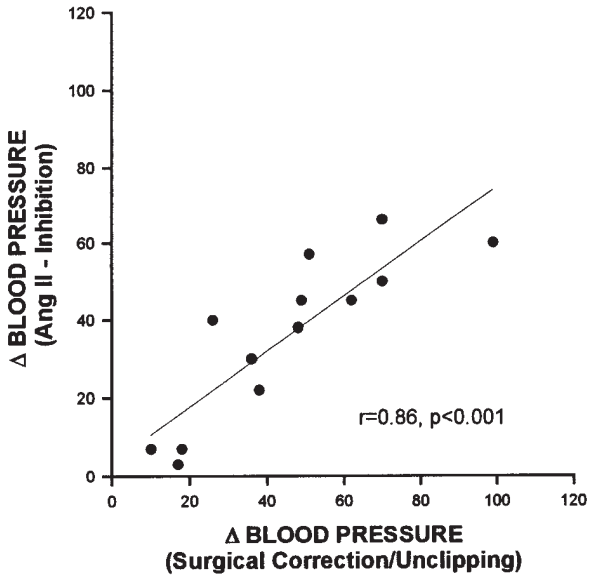

Fig. 5. Correlation between decrease in systolic blood pressure in response to 1 week of losartan treatment compared with surgical repair by means of unclipping of renal arterial stenosis among 13 rats with sustained two-kidney, one-clip renovascular hypertension.

4), from 210 ± 10 mm Hg 12 weeks after clipping to 164 ± 7 mm Hg ($p < 0.001$). The response to unclipping varied from 10 to 99 mm Hg. As with losartan administration, there was an initial rapid drop of 45 ± 6 mm Hg over the first 24 hours after unclipping (Fig. 3). This effect was not immediate, however, because blood pressure fell by only 7 ± 6 mm Hg during the first 3 hours after unclipping. The second phase of the depressor response evolved over the next 2 days, stabilizing around a mean blood pressure drop of 46 ± 7 mm Hg by day 3. Results with the indwelling arterial catheter and with the tail-cuff method correlated closely with each other ($r = 0.9$, $p < 0.001$). Among the rats subjected to sham operations, systolic blood pressure did not change significantly during the postoperative period. Mean blood pressure was only 3 ± 4 mm Hg lower than (and not significantly different from) the resting value 7 days after the procedure.

As shown in Fig. 5, a high degree of correlation between the blood pressure fall in response to 7 days of losartan treatment and the decrease after surgical repair was consistently observed ($r = 0.86$, $p < 0.001$). Thus the drop in blood pressure after 7 days of angiotensin II inhibition proved to be a reliable predictor of the extent to which blood pressure would fall after surgical repair of stenosis by means of unclipping. Plasma renin activity could not be used to predict the blood pressure response to either angiotensin inhibition with losartan or surgical unclipping in these two-kidney, one-clip rats. This finding may reflect a lack of correlation between plasma renin activity and the potential reversibility of the renovascular hypertension or the inherent variability of plasma renin activity values among anesthetized rats compared with conscious, unanesthetized rats.

Histologic evaluation

Each of the unclipped left renal arteries harvested had a patent lumen without residual narrowing at the clip site. No pathologic abnormalities were observed in the endothelium, elastic lamina, media, or adventitia (Fig. 6). The only pathologic abnormality observed at the clip site was evidence of nonconstricting periadventitial fibrosis, which did not appear to compromise the vascular lumen.

DISCUSSION

We found that rats with two-kidney, one-clip renovascular hypertension sustained over 10 to 14 weeks had near-normal rather than elevated plasma renin activity and that most rats had an attenuated blood pressure response to chronic selective angiotensin receptor blockade. This suggests that the elevated blood pressure in this sustained phase of renovascular hypertension has become, to varying degrees, relatively angiotensin independent, especially compared with the high angiotensin dependence observed in early-phase disease. Our data also suggest that the extent to which the hypertension becomes refractory to an angiotensin receptor blocker is a direct predictor of the magnitude of the

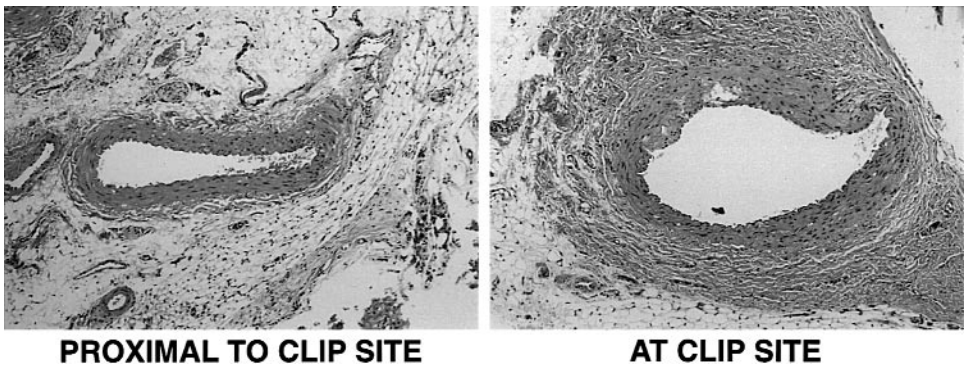

Fig. 6. Histologic comparison of the renal artery proximal to the clipping site (*left*) with the site after surgical repair by means of unclipping.

blood pressure response to surgical repair of renal arterial stenosis by means of unclipping.

Renovascular hypertension is believed to account for 5% to 10% of cases of hypertension. In general, options for direct treatment include operative revascularization by means of bypass or endarterectomy or endovascular procedures such as angioplasty with or without stent placement. As many as 20% to 40% of patients with atherosclerosis-induced renovascular hypertension exhibit an incomplete or even lack of a response despite seemingly adequate repair. This failure to improve hypertension, thereby exposing the patient to the unnecessary risk of operation, angioplasty, or stent placement may occur as the result of (1) an incorrect preoperative or preprocedural diagnosis, (2) residual or occult renovascular disease with renal hypoperfusion and continued activation of the renin-angiotensin system, or (3) progression to a chronic form of renovascular hypertension no longer remedial or responsive to repair of the stenosis. The last phase has been characterized by the potential for renal damage, vascular or endothelial dysfunction, and normal to near-normal renin and angiotensin levels.[9,15] With sustained or late-phase renovascular hypertension, blood pressure does not respond to acute single-dose intervention with ACE inhibitors or the angiotensin antagonist saralasin.[9,15] Further, the hypertension does not seem to be reversible with correction of the stenosis or removal of the ischemic kidney.[16,17] However, there is considerable controversy about the character of the so-called chronic phase. Reports have suggested varying degrees of angiotensin influence on blood pressure[21,22] as well as success of surgical intervention.[23,24] Previous studies performed on animals with sustained two-kidney, one-clip renovascular hypertension revealed inconsistent responses to either correction of the stenosis or ipsilateral nephrectomy, in that most animals continued to have hypertension.[16,17] Other investigators noted complete resolution of sustained renovascular hypertension with either unclipping or nephrectomy, whereas still others noted a depressor response to unclipping alone but not to nephrectomy.[22-24] Thus the importance of the renin-angiotensin system in sustained renovascular hypertension and, accordingly, the relation of the renin-angiotensin system to the outcome of surgical intervention remains unclear.

So what is the role of the renin-angiotensin system in the maintenance of sustained renovascular hypertension? In all probability, there is no one simple answer, and our data and the wide range of published results suggest a spectrum of possible levels of influence. In this study, plasma renin activity was 16 ± 4 ng angiotensin I per milliliter per hour among anesthetized rats with chronic two-kidney, one-clip hypertension and 11 ± 4 ng angiotensin I per milliliter per hour among rats with chronic two-kidney, one-clip hypertension subjected to sham procedures. These values are slightly but not significantly higher than those reported previously (by W.H.B.) after studies on barbiturate-anesthetized rats with normal blood pressure (10 ± 3 ng angiotensin I per milliliter per hour).[25] However, the results are only 20% to 30% of plasma renin activity values for rats with acute-phase (4 to 5 weeks) two-kidney, one-clip renovascular hypertension (51 ± 6 ng angiotensin I per milliliter per hour; $p < 0.001$).[12] Thus, notwithstanding some variability, plasma renin activity appears to be returning toward normal in two-kidney, one-clip rats as they progress away from the early phase of the disease, despite persistent hypertension.

The response to 7 days of pharmacologic angiotensin blockade was significant but only decreased blood pressure to 170 ± 8 mm Hg, thus remaining in the hypertensive range. The varying degree of the fall in blood pressure among individual rats and the lack of correlation or predictability between the response to losartan and basal plasma renin activity suggest that these two-kidney, one-clip rats 10 to 13 weeks after clipping represent a diverse population of animals undergoing change from the angiotensin dependence of the early and intermediate phases to the diminished angiotensin influence seen with sustained hypertension. Our data did not address the factors or influences that replace angiotensin in sustaining hypertension or the source of the remaining angiotensin.

In general, any hypertensive state can be seen as an inappropriate balance between the capacity of the vascular system and the volume of fluid within it. Therefore in sustained disease other vasoconstrictor systems, including the central and peripheral nervous systems, arachidonic acid metabolites, and endothelin, may be overactive and synergistic with structural abnormalities of the arterial wall, leading to an overall increase in vascular tone. These vasoconstrictors may interact with mechanisms functioning to overfill the arterial tree, such as aldosterone-induced volume retention. The source of the remaining angiotensin in chronic renovascular hypertension has not been addressed. The hypertension may originate from either the stenotic kidney or the contralateral kidney, which is unprotected and continuously exposed to this state of hypertension.[16,17,26]

Importantly, we proposed that these adaptations to sustained hypertension should be reflected by the dissipation of angiotensin influence on blood pressure. We hypothesized that changes in the role of the kidney after 10 to 14 weeks of renovascular hypertension among rats would compromise the benefits of surgical repair by means of unclipping. Because of this, we proposed that the degree to which this surgical intervention would effectively reverse hypertension could be predicted with the depressor response to 7 days of angiotensin blockade, which would serve as an index of changes taking place in the cause of the disease. It is clear from our data (Fig. 5) that there is a remarkable correlation is between the depressor response to our protocol of angiotensin blockade and the effectiveness of surgical repair by means of unclipping across a broad range of responses. The correlation is highly significant, and the absolute responses to losartan and unclipping (in millimeters of mercury) are nearly equivalent. Although our data were derived from an animal model, without the layers of complexity often accompanying human renovascular hypertension, our results reinforce the doctrine that patients with a substantial angiotensin-mediated component to their hypertension are most likely to respond to surgical therapy.

One simple explanation for the refractory responses to unclipping would be the development of structural changes in the artery after 13 to 14 weeks of being clipped, such that despite clip removal, the lumen remains compromised by retained stenosis. However, all renal arteries were dissected at the end of the protocol and no gross indication of residual stenosis was found. Further, histologic evaluation (Fig. 6) indicated evidence of only nonconstricting periadventitial fibrosis that did not compromise the vascular lumen. Thus the persistence of renovascular hypertension after clip removal must be ascribed to adaptations, both systemic and renal, that took place as hypertension evolved.

In contrast to the immediate response to ACE inhibition (30 to 60 minutes) reported for two-kidney, one-clip rats with acute hypertension (4 to 5 weeks after clipping),[12] the delayed depressor response to 7 days of losartan treatment consisted of two components—a fast phase over the first 12 hours and a slower, less pronounced phase evolving over 2 days. Other investigators have suggested that angiotensin II functions through two separate pathways: an acute vasopressor response and a chronic, slow vasopressor response.[27,28] Dickinson and Lawrence[27] first described this slowly resolving constrictor response to angiotensin in 1963, and subsequent studies have supported its existence.[27-30] In support of this, prolonged infusion of ACE inhibitors can prevent chronic renovascular hypertension[29]; established hypertension, although minimally affected by single-dose ACE inhibitors or saralasin, was partially and slowly reversed by means of chronic administration of these agents.[30] Thus continued administration of losartan (rather than a single bolus) may be critical in eliciting a complete biphasic response to angiotensin blockade. This slow pressor effect may occur as the result of a neurogenic component, increased vascular responsiveness to vasoactive agents, or alterations in the architecture of the vessel wall[28] or the renal resistance arterioles downstream from the stenosis.

In the clinical setting, most patients with renovascular hypertension are examined in the transitional or late phases of the disease.[31] The probability that some

of these patients have angiotensin-independent renovascular hypertension may be one reason for an incomplete or lack of response to surgical intervention.[4-7] Although the beneficial effects of improved blood pressure control, despite the continued need for antihypertensive agents (with reduced dosage or reduced agents) are well recognized, a reliable index to predict the degree or absence of response would be useful to establish preprocedural goals and to reduce the likelihood of a complete lack of response. The most accurate noninvasive tests in the diagnostic evaluation and potential surgical curability of renovascular hypertension are those involving a single acute dose of the ACE inhibitor captopril (the so-called captopril test). This test is used to either stimulate renin secretion (by eliminating the short-loop feedback suppression of renin by elevated angiotensin), which is then detected peripherally, or decrease angiotensin, producing characteristic changes in glomerular filtration rate in both kidneys, which are then identified with renography.[3,32] Both tests are reported to have a sensitivity of 60% to 80%, the latter permutation being slightly better.[33] Unfortunately, ACE also degrades kinins, and thus captopril may potentiate the independent depressor influence of endogenous kinin formation through stimulation of prostaglandins and nitric oxide.[3] The angiotensin antagonist saralasin has been used more recently in the diagnosis of renovascular hypertension. This compound, however, is also a partial agonist of angiotensin, and both false-positive and false-negative results have been reported without marked improvement over captopril.[3]

Although diagnostic tests with short-term administration of an ACE inhibitor are used extensively, these tests are limited as guides to surgical prognosis.[3] Besides potentiating kinins, acute administration of ACE inhibitors may not affect the slow pressor component of angiotensin II.[27-30] In keeping with our results using chronic losartan administration, it has been reported that the depressor response to long-term ACE inhibition is a more accurate predictor of surgical curability of renovascular hypertension than the response to acute single-dose tests.[34,35]

The important modifications in our method include sustained week-long (rather than short-term) use of a nonpeptide (nonagonistic) selective angiotensin AT-1 receptor antagonist (which does not influence ACE) that selectively blocks the effect of angiotensin II. Nonpeptide angiotensin II receptor antagonists are relatively new antihypertensive medications. Intravenous administration of losartan to conscious rats with normal blood pressure has no effect on blood pressure, likely reflecting the minimal role of the renin-angiotensin system in regulation of blood pressure among rats with normal blood pressure.[36] Use of this pharmacologic tool allowed us to address specifically the residual influence of angiotensin in the two-kidney, one-clip model after 10 to 14 weeks of hypertension as a predictive index of surgical success. Within the limits of our discrete animal model, a highly significant correlation (and thus predictability) was observed. Although our data suggest that the blood pressure response to long-term angiotensin II blockade can be used to predict response to surgical management of renovascular hypertension, further studies are necessary to ascertain the mechanisms that underlie the apparently angiotensin II–independent form of renovascular hypertension. Determining the cause of this persistent hypertension perhaps might be used to ascertain which patient with angiotensin-independent renovascular hypertension would be less likely to respond to surgical or endovascular manipulations.

As a cautionary note, a small subset of chronic two-kidney, one-clip rats responded to angiotensin blockade with an immediate and dramatic fall in blood pressure to hypotensive levels. It was notable that these rats did not have high plasma renin activity; if losartan was not withdrawn, the rats died within 3 days. Experience led to prestudy establishment of exclusion criteria to eliminate this subgroup from the study population. These rats perhaps represented a predominant and overwhelming angiotensin II–mediated vasoconstrictive form of hypertension with contralateral renal sodium and water excretion and resultant volume depletion. Thus, with the combination of the loss of the vasoconstrictor influence of angiotensin II and the reduction in intravascular volume, the rats could not compensate and irreversible, and often fatal, hypotension ensued. Although we do not know what separates these rats from those who can better compensate against the circulating effects of angiotensin II, this may be related to the relative level of activation of the renin-angiotensin system or differences in vascular responsiveness to angiotensin II.

In summary, we found that sustained two-kidney, one-clip renovascular hypertension in rats 10 to 14 weeks after clipping is characterized by reduced plasma renin activity compared with the high levels typically observed with acute two-kidney, one-clip hypertension. Second, in this sustained-duration model of two-kidney, one-clip renovascular hypertension, the rats exhibited an attenuated depressor response to

sustained angiotensin II blockade. The variability in blood pressure response observed with 1 week of selective angiotensin II receptor blockade correlated with a subsequent response to surgical repair by means of unclipping of the renal arterial stenosis. We propose that the elevated blood pressure in this sustained model of renovascular hypertension has become, to varying degrees, relatively angiotensin independent. The extent to which this chronic hypertension becomes refractory to intervention with angiotensin receptor blockers is a direct predictor of the potential for success of surgical repair of renal arterial stenosis in lowering elevated blood pressure.

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